Purpose:Making solutions for gels for protein analysis and characterization

Make this the day before or day that you want to run the gel.

Important Notes:

\*Measure all solutions in graduated cylinders. Volume markings on bottles are NOT accurate.

\*SDS bubbles…make sure when measuring volume to look at the level of the liquid under the bubbles.

**Stock Solutions for Polyacrylamide Gels**

1. **40% acrylamide: bisacrylamide (37.5:1**)– stored at 4°C; NEUROTOXIN!!!
2. **1 M Tris-HCl, pH 8.8:**
   1. Measure 60.57 g Tris base (121.14 g/mol)
   2. Fill up to ~400 mL with Nanopure H2O and stir until dissolved
   3. Add conc. HCl until pH 8.8
   4. In graduated cylinder, add Nanopure H2O to give total volume 500 mL
   5. Store in 4°C
3. **0.5 M Tris-HCl, pH 6.9:**
   1. Measure 15.14 g Tris base (121.14 g/mol)
   2. Fill up to ~200 mL with Nanopure H2O and stir until dissolved
   3. Add conc. HCl until pH 6.9
   4. In graduated cylinder, add Nanopure H2O to give total volume 250 mL
   5. Store at 4°C
4. **10% Ammonium Persulfate (Discard after a week):**
   1. In a 1.7 mL microcentrifuge tube, add 20 mg ammonium persulfate
   2. Add Nanopure H2O to give total volume of 200 uL
   3. Store in -20°C
5. **10% SDS**
   1. Measure 10g SDS
   2. Add Nanopure H2O to give total volume of 100 mL
   3. Store at room temperature

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**Assemble gel hardware and drying stand**

Two glass plates – one notched

Two spacers (white skinny rectangular)

One big white spacer (temporary use)

Gel pouch (plastic)

Blocking plate (clear thick) and drying stand

Well comb (white with teeth)

**Make 12% Resolving/Separating Gel: (make before stacking gel)**

|  |  |
| --- | --- |
|  | **Resolving (separating)** |
| **H20** | 2.15 **ml** |
| **1M TrisHCl pH 8.9** | 1.25 **ml** |
| **0.5 M TrisHCl ph 6.8** | 0 |
| **10%SDS** | 50 ul |
| **10% APS** | 31 ul |
| **40% acrylamide:bisacrylamide** | 1.5 **ml** |
| **TEMED** | 10 ul |
|  | **5 ml total volume** |
| This is enough for 1 gel that is 12% | |

**(This is the bottom gel)**

Mix components in a small beaker

\*Make sure you have Pasteur pipet or pipetter ready after adding APS and TEMED because acrylamide can polymerize quickly!

After pipetting enough gel (about ¾ of plate from the bottom), add butanol or 100% ethanol (found in flammable cabinet) to level out top of gel.

When gel is all polymerized – 30 min – 1hr, wash out the alcohol from the top with water.

Then proceed to make stacking gel.

|  |  |
| --- | --- |
|  | **Stacking** |
| **H20** | 1.58 **ml** |
| **1M TrisHCl pH 8.9** | 0 |
| **0.5 M TrisHCl ph 6.8** | 625 ul |
| **10%SDS** | 25 ul |
| **10% APS** | 25 ul |
| **40%**  **acrylamide:bisacrylamide** | 250 ul |
| **TEMED** | 5 ul |
| Bromophenol blue powder to help visualize wells | **1 grain** |
|  | **2.5 ml total volume** |

**Make 5% Stacking Gel:**

**(This is the top gel)**

Mix components in a small beaker

\*Pipette immediately and add 10-well comb to gel.

Add gel to top

Let the gel polymerize

Once dried, store with combs in place in 4OC. Wrap in saran wrap.

Label with tape and your name and date.

Discard gel solution mix and dried mix in black gel waste tubs